An *In Silico* Approach to Analyse Imatinib Analogues as Effective Protein Kinase Inhibitors against BCR-ABL in Chronic Myeloid Leukemia

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By
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CERTIFICATE

This is to certify that the thesis entitled **"An *In Silico* Approach to Analyse Imatinib Analogues as Effective Protein Kinase Inhibitors against BCR-ABL in Chronic Myeloid Leukemia"** by **Biswaprakash Ojha (110BM0013)** submitted to the National Institute of Technology, Rourkela for the Degree of Bachelor of Technology is a record of bonafide research work, carried out by him in the Department of Biotechnology and Medical Engineering under my supervision and guidance.To the best of my knowledge, the matter embodied in the thesis has not been submitted to any other University/ Institute for the award of any Degree or Diploma.

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ABBREVIATIONS

PDB: Protein data bank

TKI: Tyrosine kinase Inhibitor

AP: Accelerated phase

CP: Chronic phase

BP: Blastic phase

CGgR: Cytogenic response

TRP: Tryptophan

Asp: Aspartic acid

Thr: Threonine

Phe: Phenylalanine

Lys: Lysine

Gln: Glutamine

His: Histidine

Arg: Arginine

ADME: Absorption Distribution Metabolism Excretion

CML: Chronic Myeloid Leukemia

BMT: Bone Marrow Transplant

INF: Interferon

ABSTRACT

Chronic myelogenous leukemia (CML), is a heterogeneous clonal myeloproliferative disorder, which occurs from the neoplastic transformation of the primitive hematopoietic stem cell. The presence of a balanced translocation t(9;22) (q34;q11), known as the Philadelphia (ph) chromosome which is the basis of the diagnosis and the hallmark of the treatment. The chromosomal translocation results in the reciprocal fusion of the BCR to the ABL gene to form the chimeric BCR-ABL gene. The respective oncoprotein (BCR-ABL) exhibits constitutively activated tyrosine kinase activity that is responsible for the activation of signal transduction pathways that lead to the abnormal bone marrow proliferation and to the clinical and morphologic manifestations of this unique leukemia. CML accounts for 20% of all leukemias affecting adults. Imatinib, which specifically inhibits the BCR-ABL kinase was first introduced into practice in 2001. Although it is established as a long term effective therapy for patients with chronic myeloid leukemia (CML), the research continues to advance on developing second generation tyrosine kinase inhibitors such as Dasatinib, Nilotinib. The current project has focused on computational analysis of protein kinase inhibitors that target the kinase domain of BCR-ABL which are structurally related to Imatinib. The protein kinase inhibitors was assessed for the inhibition property against CML from the Protein Data Bank (PDB). Total 53 ligands were structurally optimized and docked against the CML target proteins BCR-ABL in Autodock vina. The ligand with significant higher binding energy compared to Imitinib were validated for drug likeness on ADMET toxicity screening tool. Intriguingly all the fatty acids showed docking energy in the range of -3.0 to -9.1 Kcal/mol. Among the different ligands, Alvocidib exhibited best affinity for the target as evident by the strong interaction with the target proteins in Ligplot. The *in silico* ADME results further substantiated the efficacy of Alvocidib showed as a potential natural source which could inhibit the tyrosine kinase activity of the BCR-ABL.

Keywords: Chronic Myeloid Leukemia (CML), BCR-ABL, Protein Kinase Inhibitors, PDB, Ligands

CHAPTER 1: INTRODUCTION

1.1 LEUKEMIAS

Leukemia is a cancer of the blood-forming tissues characterized by the uncontrolled accumulation of immature white blood cells called "blasts" in the tissues of the body with or without a corresponding increase of those in the circulating blood. Leukemia is a broad term covering a group of diseases which have an effect on the blood, bone marrow, and lymphoid system, are all known to be hematological neoplasms [1].

Each year there are 351,000 new cases of leukemia worldwide which represent 2.8% of all cancers and 3.4% of deaths from cancer [2]. The number of people in the US are living with, or are in remission from, leukemia are estimated to 310046 [3]. It accounts for 30% of all cancers diagnosed in children under 15 years of age in industrialized countries [4].

Tabele 1. Prevalence of leukemia.

Approximate US Prevalence of the Four Major Types of Leukemia as of January 1, 2010	
Type	Prevalence
Acute Lymphoblastic Leukemia	66,030
Chronic Lymphocytic Leukemia	119,386
Acute Myeloid Leukemia	35,726
Chronic Myeloid Leukemia	31,586

Table 1. Source: Surveillance, Epidemiology, and End Results (SEER) Program (www.seer.cancer.gov). Prevalence database: "US Estimated 35-Year L-D Prevalence Counts on 1/1/2010". National Cancer Institute, DCCPS, Surveillance Research Program, Data Modeling Branch, released April 2013, based on the November 2012 SEER data submission.

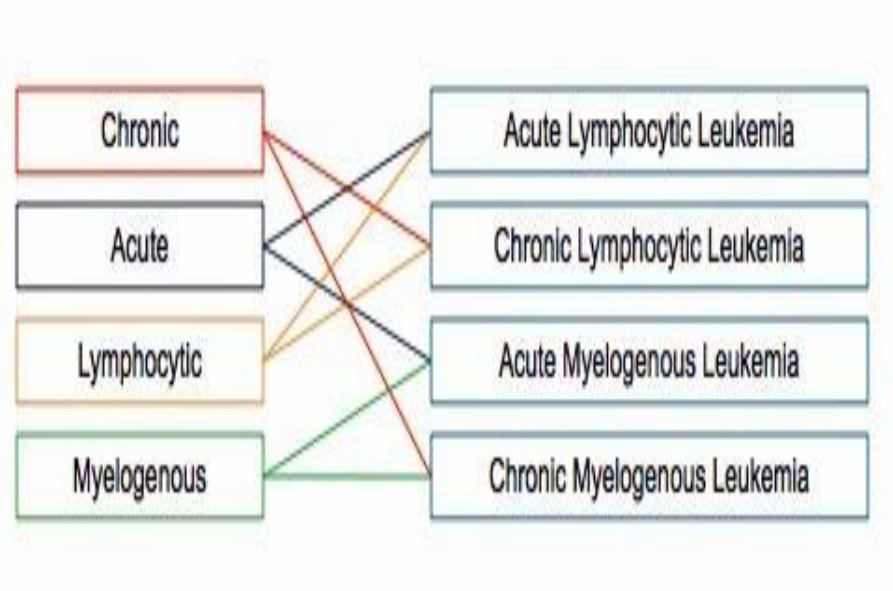


Figure 1. Leukmia Classification

Table 2. French-American-British (FAB) Classification of Acute Myeloid Leukemia (AML).

FAB TYPE	DESCRIPTION
M0	Minimally differentiated AML
M1	Myeloblastic leukemia with little maturation
M2	Myeloblastic leukemia with maturation
M3	Acute promyelocytic leukemia (APL)
M3h	APL, hypergranular variant
M3v	APL, microgranular variant
M4	Acute myelomonocytic leukemia (AMML)
M4e0	AMML with dysplastic marrow eosinophils
M5	Acute monoblastic leukemia (AMoL)
M5a	AMoL, poorly differentiated
M5b	AMoL, differentiated
M6	Erythroleukemia
M6a	AML with erythroid dysplasia
M6b	Erythroleukemia
M7	Acute megakaryoblastic leukemia (AMkL)

1.2 TYPES OF LEUKEMIA

Leukemias can be classified in different ways based on the course of the disease and the predominant type of white blood cell involved. The most common types of leukemia are categorized in to four ways:

1. **Acute myeloid leukemia (AML)**
   AML affects myeloid cells and grows quickly. About 18,000 new cases of this type of leukemia is enrolled each year. It occurs in both adults and children. AML is a common type of leukemia. AML is the most common type of acute leukemia in adults and affects males significantly more often than females.
2. **Acute lymphoblastic leukemia (ALL)**
   ALL affects lymphoid cells and grows quickly. This is the most common type of leukemia in young children. Adults over the age of 65 are mainly affected by this type of leukemia. It accounts for more than 6,000 new cases of leukemia each year.
3. **Chronic myeloid leukemia (CML)**
   CML affects myeloid cells and usually grows slowly at first. The abnormal blood cells work okay. It accounts for nearly 6,000 new cases of leukemia each year. It mainly affects adults. 90% of treated patients survive for over 5 years. A person with CML may have few or no symptoms for months or years before entering a phase in which the leukemia cells grow more quickly.
4. **Chronic lymphocytic leukemia (CLL)**
   CLL affects lymphoid cells and usually grows slowly. The abnormal cells work almost as well as the normal white blood cells so it feels well for years without needing any treatment. It accounts for more than 15,000 new cases of leukemia each year. It almost never affects children, most often diagnosed patients are above age of 55. Over 60% of major patients with CLL are men. 75% of treated CLL patients survive for over five years. Experts say it is incurable. A more aggressive form of CLL is B-cell prolymphocytic leukemia.

Each main type of leukemia is named according to the type of cell that's affected (a myeloid cell or a lymphoid cell) and whether the disease begins in mature or immature cells. The terms “myeloid” or “myelogenous” and “lymphoid,”“lymphocytic” or “lymphoblastic” denote the cell types involved. If the cancerous transformation occurs in the type of marrow that makes lymphocytes, the disease is called lymphoid, lymphocytic, or lymphoblastic leukemia. If the

cancerous change occurs in the type of marrow cells that go on to produce red blood cells, other types of white cells, and platelets, the disease is called myeloid, myelogenous, or myeloblastic leukemia. Based on how quickly the leukemia develops and grows it can be Acute, which is a rapidly progressing disease that results in the accumulation of immature, useless cells in the marrow and blood, or Chronic, which progresses more slowly and allows more mature, useful cells to be made. Adults can get either type; childen with leukemia most often have an acute type. Other types of leukemia and related disorders include:

1. Hairy cell leukemia
2. Chronic myelomonocytic leukemia (CMML)
3. Juvenile myelomonocytic leukemia (JMML)

1.3 CHRONIC MYELOID LEUKEMIA

Chronic myelogenous leukemia (CML), also known as chronic myeloid leukemia, is a heterogeneous clonal myeloproliferative disorder, which occurs from the neoplastic transformation of the primitive hematopoietic stem cell. It is a type of leukemia that is characterized by increased proliferation of the granulocytic cell line without the loss of their capacity to differentiate. It accounts for 20% of all leukemias affecting adults with an estimated 5920 new cases and 610 deaths due to this disease in the United States (US) in 2013 [34].

The pathologic hallmark of this disease is the Philadelphia chromosome which is observed in about 90% of CML patients [37]. The disease is associated with a chromosomal abnormality, this genetic abnormality results from translocation of ABL1 from chromosome 9q34 with the breakpoint cluster region (BCR) gene on chromosome 22q11.2. This rearrangement is known as the Philadelphia chromosome. The molecular consequence of this translocation is the generation of a BCR-ABL fusion oncogene, which in turn translates into a BCR-ABL oncoprotein. The respective oncoprotein (BCR-ABL) exhibits constitutive activated tyrosine kinase activity that is responsible for the activation of signal transduction pathways that lead to the abnormal bone marrow proliferation and to the clinical and morphologic manifestations of this unique leukemia [36]. CML can be divided into three phases: the chronic phase (CP), the accelerated phase (AP), and the blast phase (BP) that resembles acute leukemia.

1.4 PHILADELPHIA CHROMOSOME

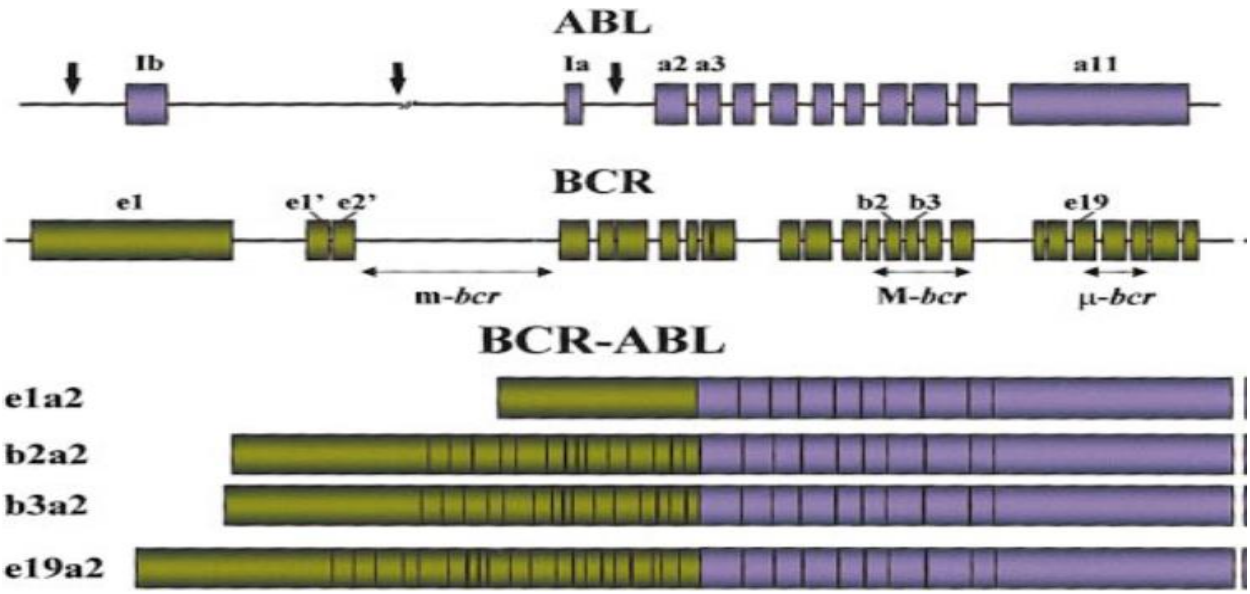


Figure 2. Locations of the breakpoints in the ABL and BCR genes and structure of the chimeric mRNAs derived from the various breaks Locations of the breakpoints in the ABL and BCR.

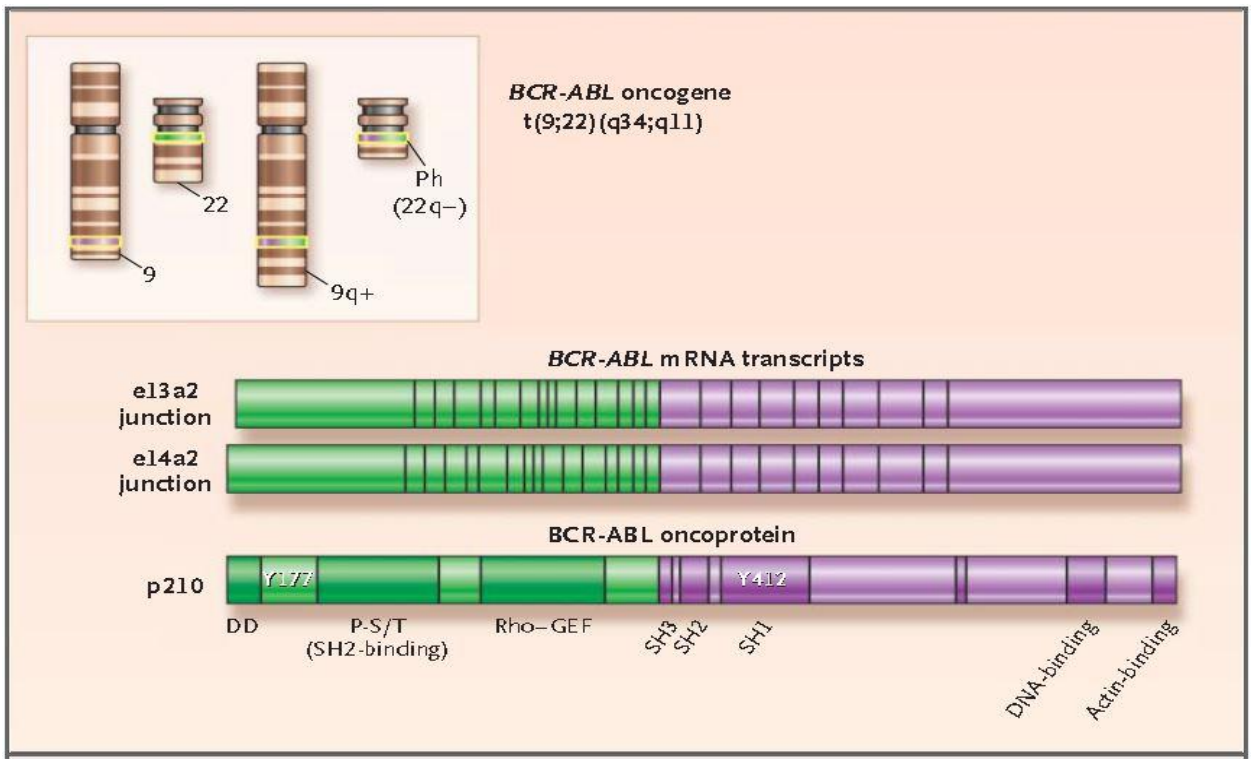


Figure 3. The t(9;22) Translocation and Its Products: the BCR-ABL Oncogene on the Ph Chromosome and the Reciprocal ABL-BCR on the Derivative 9q+ Chromosome.

The translocation between chromosomes 9 and 22 t(9;22)(q34;q11) or its variants t(V;9;22) [1]. This reciprocal translocation generates the shortened 22q known as the Philadelphia (Ph) chromosome and the new fusion oncogene is called as BCR-ABL (Breakpoint Cluster Region-Abelson Leukemia). This oncogene encodes a chimeric 210 KD BCR-ABL protein that incorporates an activated ABL tyrosine kinase domain. The constitutive activity of this tyrosine kinase plays a central role in the pathogenesis of the disease. The expression of chimeric BCR-ABL protein with deregulated tyrosine kinase activity has been shown to be necessary and sufficient for the trans-formed phenotype of CML cells. Most of the CML patients have been diagnosed in chronic phase (CP), but as a result of genomic instability, it progresses to ill-defined unstable accelerated phase (AP) and then to the terminal blastic crisis phase (BP) over time, becoming increasingly resistant to therapy.

1.5 MECHANISM OF BCR ABL SIGNALLING PATHWAYS

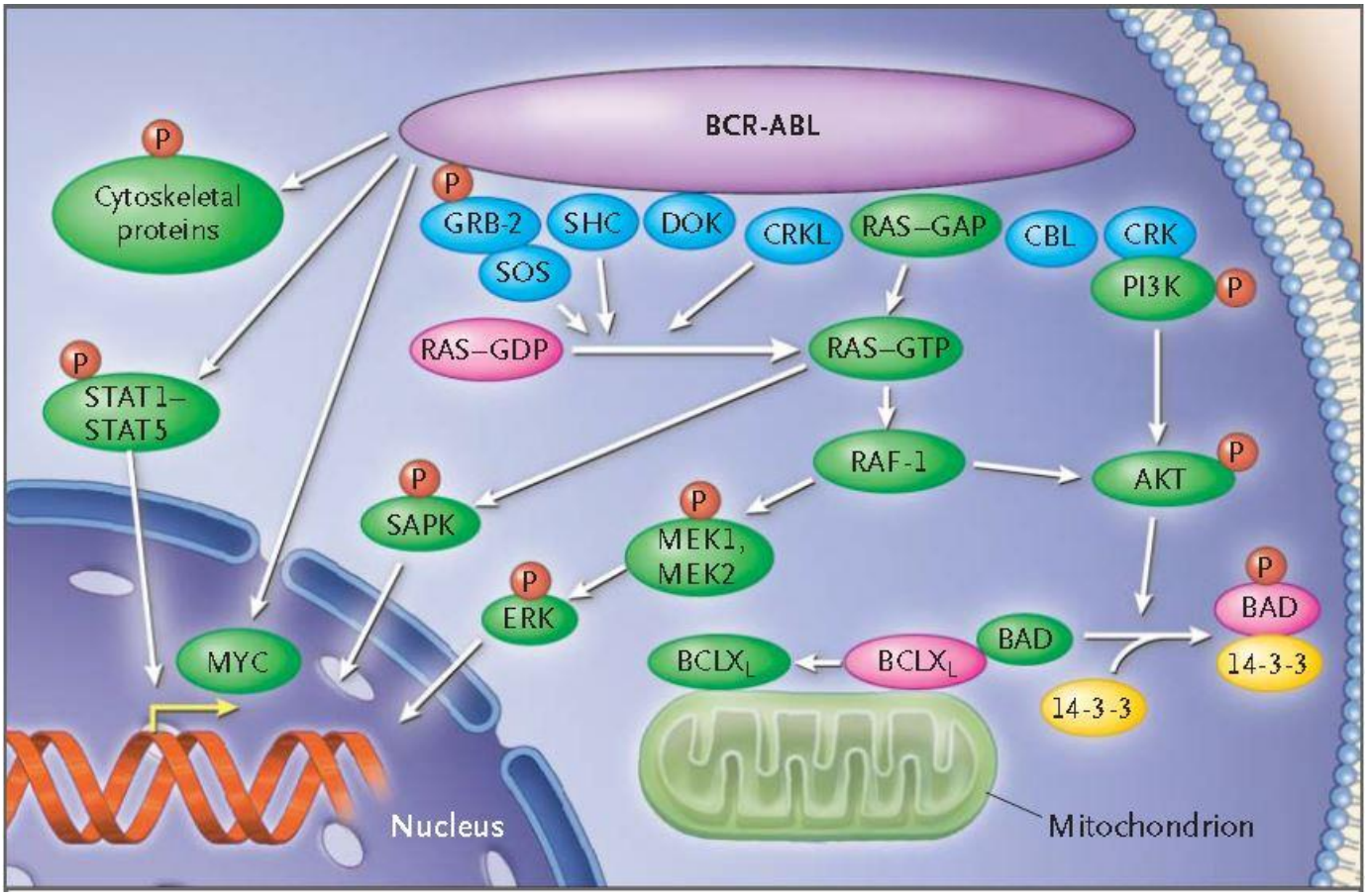


Figure 4. Signal Transduction Pathways Affected by BCR-ABL.

1.5.1 RAS-RAF-MAPK-ERK PATHWAY

Leads to the activation of gene transcription and Ras activation is important for pathogenesis of Ph-positive leukemias.

1.5.2 MYC PATHWAY

On the cellular context, Myc constitutes a proliferative or an apoptotic signal. The apoptotic arm of its dual function is counterbalanced in CML cells by other mechanisms, such as the Phosphatidilinositol-3-kinase (PI3k) pathway.

1.5.3 PHOSPHATIDILINOSITOL -3-KINASES (PI3KS) PATHWAY

PI3 kinase activity is required for the proliferation of BCR-ABL positive cells. The final process potentially induces more efficient degradation of p53 through p110.

1.5.4 JAK-STAT PATHWAY

STAT5 functions as a transcription factor. The activation of STAT5 by p210 BCR-ABL induces malignant transformation of the K562 cell and inhibits apoptosis by up regulating the transcription of BCL-XL.

1.5.5 CYTOSKELETON PROTEINS

The function of β-integrins on the surface of CML cells is abnormal and the final effect is reduced adhesion and increased proliferation.

1.5.6 TRANSFORMATION

The BCR-ABL abrogates cell dependence on external growth factors by up-regulating interleukin production and alters the cell adhesion properties by modulating the expression and activation of focal adhesion kinase and other proteins.

1.6 OBJECTIVE

- To explore the agents that are linked to BCR-ABL and its receptors, central to transduction pathways responsible for CML.
- To identify the ligands from the pool of protein kinase inhibitors of BCR-ABL with structural similarity to Imitanib by binding energy estimation.
- To analyze the suitability of the novel inhibitors as drugs against CML by ADMET studies.

CHAPTER 2: LITERATURE REVIEW

2.1 THERAPEUTIC OPTIONS CML

Table 3. Currently available Therapies for Chronic Myeloid Leukemia.

AGENT/THERAPY	CLASS OF AGENT	EFFECT ON DISEASE
Hydroxyurea	Antimetabolite	Rapid cytoreduction
Interferon alfa	Immune modulator	10%–38% major cytogenetic response, Lessening of effect over time, Safe during pregnancy
Imatinib	Tyrosine kinase inhibitor (TKI)	Now treatment of choice; 70% long-term disease control at molecular level
Dasatinib, nilotinib	Second generation TKI	Mostly used as second-line therapy in imatinib failure or intolerance
Allogeneic bone marrow transplantation	Not applicable	Remains the only known cure; Generally now reserved for TKI failure

2.1.1 NON-TARGETED THERAPY

Busulphan or hydroxyurea were treated in the chronic phase (CP) of the disease in over 80% of patients. In CP, generally the median survival was 35 to 65 months. Before the development of blastic phase (BP) starts, two thirds of patients will progress to the accelerated phase (AP). The median survival for patients with AP is 1 to 2 years and for BP patients is 3 to 12 months. But when patients are treated with interferon (IFN), severe toxicities and reduction in efficacy, persistence in CP disease and progression are mainly associated with it. So in this treatment the median survival of patients was 65 to 90 months and complete cytogenetic responses (CCgR) at rates of 5 to 20% in early CP CML [5]. In the CML 91 trial 325 patients were included in the period of 1991 to 1996 and compared with 553 patients in the Imatinib (IRIS: International Randomized Imatinib Study) group between 2001 to 2002. Both groups of patients, showed CCgR, survival free of transformation for a follow-up of 42 months. Overall survival rates were considerably higher with Imatinib compared than with IFN/Ara-C ($p<.001$, $p= .004$, and $p<.001$ respectively). So this historical comparison is relevant because the survival

benefit in the IRIS study for IFN/Ara-c could not be evaluated due to the high crossover rate to the Imatinib group. Allogeneic stem cell transplantation is a potential curative for leukemia although it is limited by suitable donor availability and transplant associated mortality and morbidities. 427 abstract were presented in a randomized study of 621 new patients in CP Ph+ with a follow-up of 8 to 9 years. The result form this follow up is that, those patients who are treated with the best available drug treatment (first treated with INF alpha and Gleevec thereafter) the median survival was 73%. But the median survival is 62% for those who are treated with allogeneic stem cell transplant (p=0.049). From this experiment they concluded that in particular for those patients with low risk 85% vs. 68% respectively, Drug treatment is superior to hematopoietic stem cell transplantation (HSCT) in patients with CP CML. Transplant related mortality (26.1%) is reflected by inferior survival rate in the transplant group [7].

2.1.2 TARGETED THERAPIES

The most important basis of the pathophysiology of the CML is the understanding of the signaling molecular pathways of the BCR-ABL cells which was the hallmark of new drug research. All cellular events are governed by signal transduction events that depend on highly coupled intracellular networks of specific protein-protein interactions, which are, in turn, functionally controlled by reversible phosphorylation reactions catalyzed by protein kinases. Accordingly, Tyrosine kinases play principal role in initiation of signal transduction pathways in CML. The common feature preserved in protein kinase family is the catalytic domain with its associated catalytic center. Almost all proteins kinases employ ATP as a co substrate in order to transfer the gamma-phosphate of ATP onto an acceptor protein, peptide or lipid substrate [8]. The chimeric oncogene BCR-ABL that is generated by the fusion of BCR and ABL is not found in normal cells. The respective oncoprotein BCR-ABL, which exhibits constitutively tyrosine kinase activity leading to excessive cell proliferation. The drugs that inhibit BCR-ABL activity known as tyrosine kinase inhibitors (TKIs) are mainly the standard cure for CML. These drugs are expected to act as targeted therapy as they don't affect normal cells. So they don't have severe side effects as seen with other drugs which are used for treatment of CML like Traditional chemotherapy drugs or interferon. But it is of great importance of understanding that if the TKIs are taken during pregnancy, they can harm the fetus. TKIs appear as the best drug against chronic phase CML, but there are other newer drugs which can treat patients with more advanced disease [10].

2.1.2.A Imatinib

In a program involving investigation of the development of specific TKIs, Imatinib or STI571 [signal transduction inhibitor, (formerly known as CGP 57148B)] was generated [9]. Imatinib is a 2-phemylamino-pyrimidine, which is an oral administered drug and exhibits tyrosine kinase inhibition once a day. The BCR-ABL protein which signals the body to proliferate too many WBCs is inhibited by Gleevec by binding to the kinase domain of the receptor. It blocks the pathway initiated by BCR ABL and stops the overproduction of WBCs. It was revealed that the cellular proliferation and apoptosis induction of BCR-ABL CML and ALL cells lines are blocked by the drugs activity. Normally tyrosine kinase helps to stimulate the growth of cancer cells. Blocking tyrosine kinase reduces the production of abnormal white blood cells. Gleevec turn out to be the first standard cure for CML patients as it particularly inhibiting the BCR-ABL protein. As it was the first TKI drug used against CML, so it is called as first generation TKI. In some patients, imatinib resistance occurs in which the drug ultimately stop working. Resistance occurs due to the formation of mutation gene from the normal gene which can be overcome by dose enhancement. But sometimes patients have to shift to other drugs which are of grater potency like 2^{nd} generation TKIs. As it slows the development of the leukemia and prevent the condition reaching to AP phase so it is given on completion of diagnosis [10]

2.1.2.B Nilotinib

Nilotinib (AMN107, Tasigna™; Novartis) is discovered by the incorporation of an amide (N-methylpiperazine) moiety into imatinib to enhance its solubility and oral bioavailability. Replacement of the amide moiety with alternate binding groups and also retaining hydrogen bond interactions to Glutamine286 and Aspartase381, led to the formation of a more potent structural derivative of imatinib which is an amino pyrimidine [11]. This drug binds to the inactive conformation of the kinase domain of BCR-ABL protein, with a 25-fold increase potency as compared to Imatinib molecule. It mainly targets the most resistance shown by mutational change of gene in imatinib treatment except T315I mutation. PDGFR (Platelet Derived Growth Factor Receptor) and c-Kit are inhibited by the activity of nilotinib drug but have no effect on Src kinases [12-14]. Hematologic and cytogenetic responses were noted as 89% and 50% respectively in CP CML patients that are resistant to imatinib treatment. The response rates are dependent on drug dosage of nilotinib which is generally 400mg twice a day.

Accelerated, myeloid and lymphoid blastic phases of CML were also promised to have a positive effect in patients by this results [15-18].

2.1.2.C Bosutinib

Bosutinib is one of the most capable drugs among other second line treatments available for CML. It was found that Bosutinib is more effective than imatinib, and it can overcome several number of mutations that reduce CML resistant to imatinib. Although bosutinib is better than many other existing treatments for CML, it is may not be effective for all patients. The T315I point mutation which can developed by BCR-ABL protein in CML renders resistant to imatinib, bosutinib, and other TKIs. Bosutinib is effective against the patients, those were treated by the first and second generation TKI and shown resistance to the treatment, as it is a 3rd generation TKI. Unlike imatinib, Both ABL and Src kinases autophosphorylation are inhibited by bosutinib, and resulted in reticent of cell proliferation and apoptosis. So this drug is approved by Food and Drug Administration in United States for the treatment in chronic, accelerated, or blast phase in patients resistant or intolerant to other TKIs.

2.1.2.D Dasatinib

Some of the multi-target kinase inhibitors of BCR-ABL, ephrin receptor kinase, SFK and PDGFR is Dasatinib (BMS-354825, Sprycel; Bristol Myers Squibb). Dasatinib is not related to Imatinib in structure. Dasatinib is a thiazolecarboxamide. Dasatinib has the capability of binding to both active and non-active conformational sites of ABL kinase domain. In addition to that this molecule can inhibit Src kinase which targets different tumor progressive pathways relative to that of Imatinib molecule [19]. Research says that Dasatinib have 325 fold of increased potency as compared to Imatinib and capable of harboring potent inhibitory activities against Imatinib resistance mutant tests [20]. Sprycel is a TKI (Tyrosine Kinase Inhibitor) which targets BCR ABL protein. It is also called as second generation of TKI as it comes after Imatinib. Sprycel is an oral bio-available drug. Dasatinib is used as the drug for initial treatment for CML; it acts as a replacement drug for patients who can’t take Imatinib as a drug for CML. During the approval of this drug it was said to take twice a day as a pill, but more often larger dose is preferred for a single day. Dasatinib more often inhibit CrkL phosphorylation and result in reduction of total number of CD34+CD38– CML cells as compared to Imatinib.

2.1.2.E Omacetaxine Mepusuccinate

Adult patients with more than one resistance or intolerance history while earlier therapies (tyrosine kinase inhibitors) are recommended Synribo or Omacetaxine Mepusuccinate. It is generally administered subcutaneously and is found effective in patients with history of multiple tyrosine kinase inhibitor failures. It is injected subcutaneously and is effective in patients with multiple tyrosine kinase inhibitor failures, also with the T315I mutation. Synribo was approved on the basis of the responses of the volunteers during the trial. The patients were both in chronic and accelerated phase. The data for long term survival is still being accrued.

2.1.2.F Interferon Alpha

Interferon is one of a kind of biological therapy that can execute well for chronic phase CML. It is usually can be used in the following situations;

- If other types of biological therapy do not work or stop working after a while
- If other types of biological therapy cause too many side effects
- If you had a bone marrow or stem cell transplant but your CML comes back.

About 1 out of 5 people shows such responses that are so good that there are no longer any Philadelphia chromosome positive cells present in their bloodstream or bone marrow. Usually this response is long lasting and sometimes it works for 10 years or more. Usually people carry on with the interferon for 2 or 3 years at the least. Interferons have various side effects but these side effects vary a lot in different people. Some people don't have any or a little trouble with interferon. Others face various troubles with interferons. Some of the most common side effects of this are flu like reaction, with aching muscles, a high temperature and weakness. In these situations Paracetamol usually helps. Some people may also feel nauseating and lose their appetite.

2.1.2.G Ponatinib

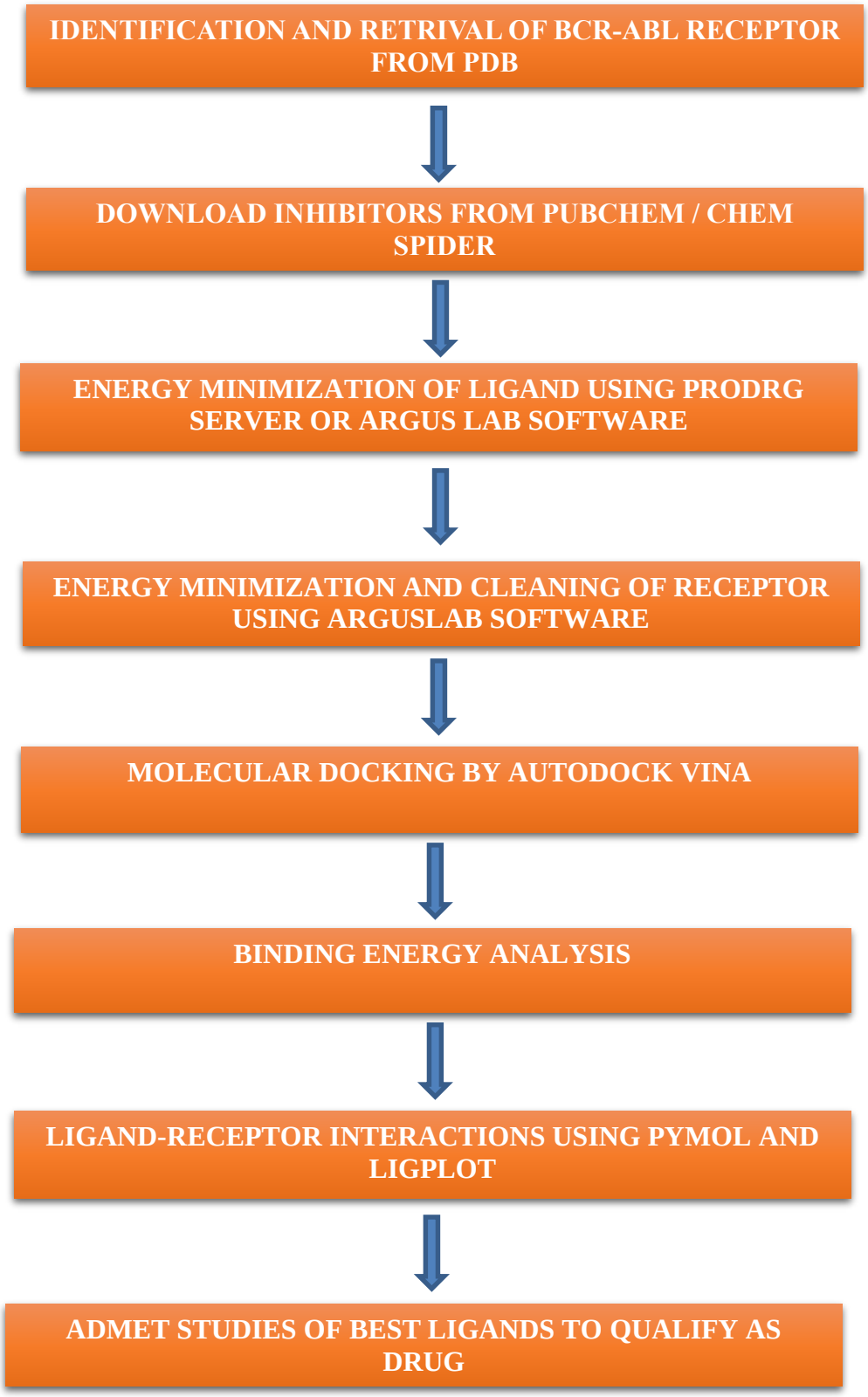
Ponatinib is a second generation tyrosine kinase inhibitor targeting BCR-ABL, so it is used in patients with CML after the treatment with another TKI. When the drug resistance appears in patients due the mutation of the gene, it is effective than other TKIs. The mutation developed due to the treatment of TKI in some patients, is known as T315I mutation. First generation TKIs are ineffective against this type of mutation. But ponatinib is the first potent tyrosine kinase inhibitor which remains effective against all other known BCR-ABL permutations and target T315I point mutation in CML. In spite of side effects as other TKIs, it remains as a promising drug in the treatment of CML by targeting the intractable T315I mutation. Ponatinib was recently approved by Food and Drug administration in United States as a second line cure for CML in patients who have shown resistance to multiple other TKIs due to its effectiveness against T315I mutated BCR ABL. It is an oral bioavailable drug prescribed to take a pill once a day.

2.1.3 BONE MARROW AND STEM CELL TRANSPLANTS

Long term remission in CML can only achieved by bone marrow or cord blood transplant (BMT) of stem cells from a related or unrelated donor. Stem cells of different organisms are involved in this type of treatment so these types of transplants are known as allogeneic transplants. Another type of transplant, in which patient's own cells are used in the bone marrow transplantation is known as autologous transplant. These are hardly ever used in the treatment of CML. High dose chemotherapy is given to the patient to kill the cancerous cells already present before the bone marrow transplantation. Then the bone marrow of patient is infused and repopulated with the transplant cells. A sibling or a twin is often the best match. Bone marrow transplants are prone to risks and side effects and potential complications. In most cases of chronic leukemia especially among elderly patients, the potential risks of transplantation far outweigh any benefit. The risks associated in this treatment are;

- Graft versus host disease (GVHD).
- Veno occlusive disease.
- Life threatening infections.
- Risk of secondary malignancies.

CHAPTER 3: PLAN OF WORK



CHAPTER 4: MATERAILS AND METHOD

4.1 TOOLS USED

- **Marvin Sketch** - For drawing Novel Ligand/Inhibitor
- **Online Translator -** For Translating ligand file format to pdb
- **PDB -** For Downloading Receptor/Protein .pdb files
- **Corina-** To conver 2d ligands to 3d
- **Arguslab softaware-** For cleaning and minimization
- **Pubchem** – Database of inhibitors
- **Prodrg Server-** Energy minimization of inhibitors
- **Chimera** - For Visualization and Energy Minimization studies
- **Openbabel-** To convert to different formats
- **Pymol** – For visualization and alignment
- **Auto Dock 4** - Potential offline Docking(protein+ligand) tool widely used
- **Autodock vina** - Docking without grid box
- **Ligand scout** - To find the specific interactions
- **Lig plot –** To find out atomic interactions
- **FAF-DRUG-** To check drug likeness

4.2 RETRIEVING RECEPTORS FROM PDB

- Open Protein data bank, rcsb.org.
- Type receptor name in search i.e. BCR-ABL.
- Retrieve the pdb (3CS9) files of the receptors and store them in pdb.text file format for further use.

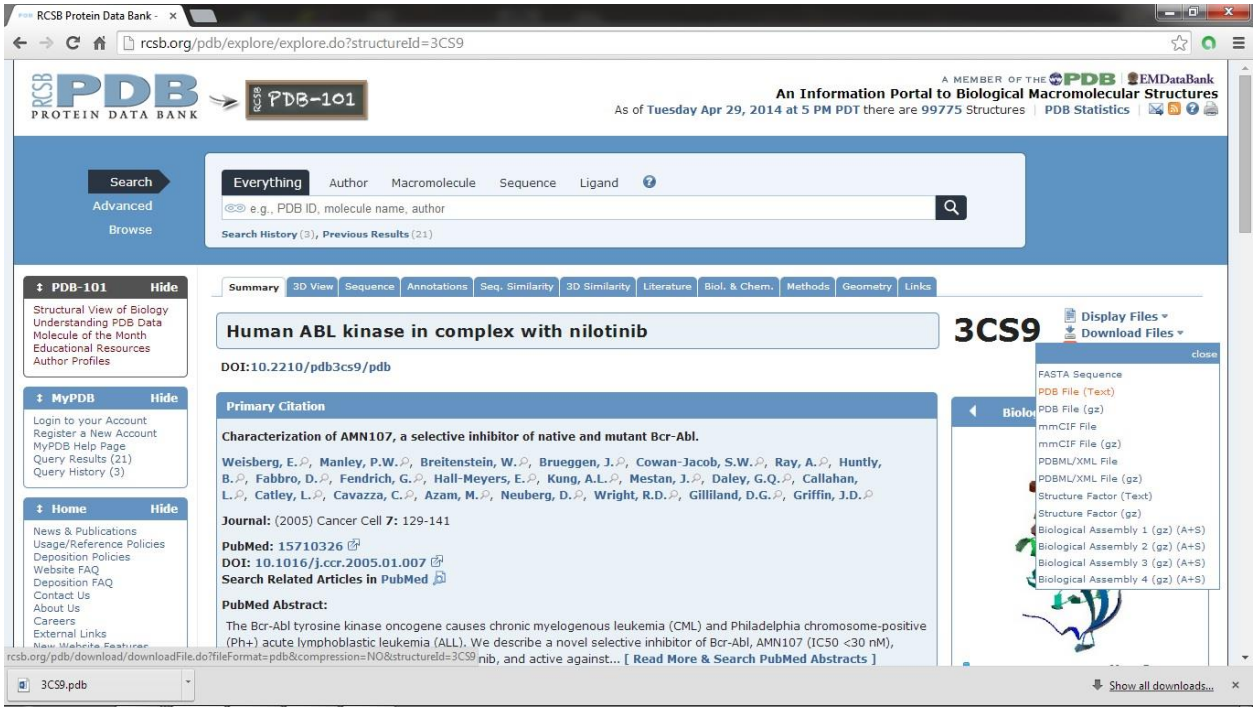


Figure 5. Snapshot of Protein Data Bank webpage with BCR-ABL receptor (PDB ID: 3CS9).

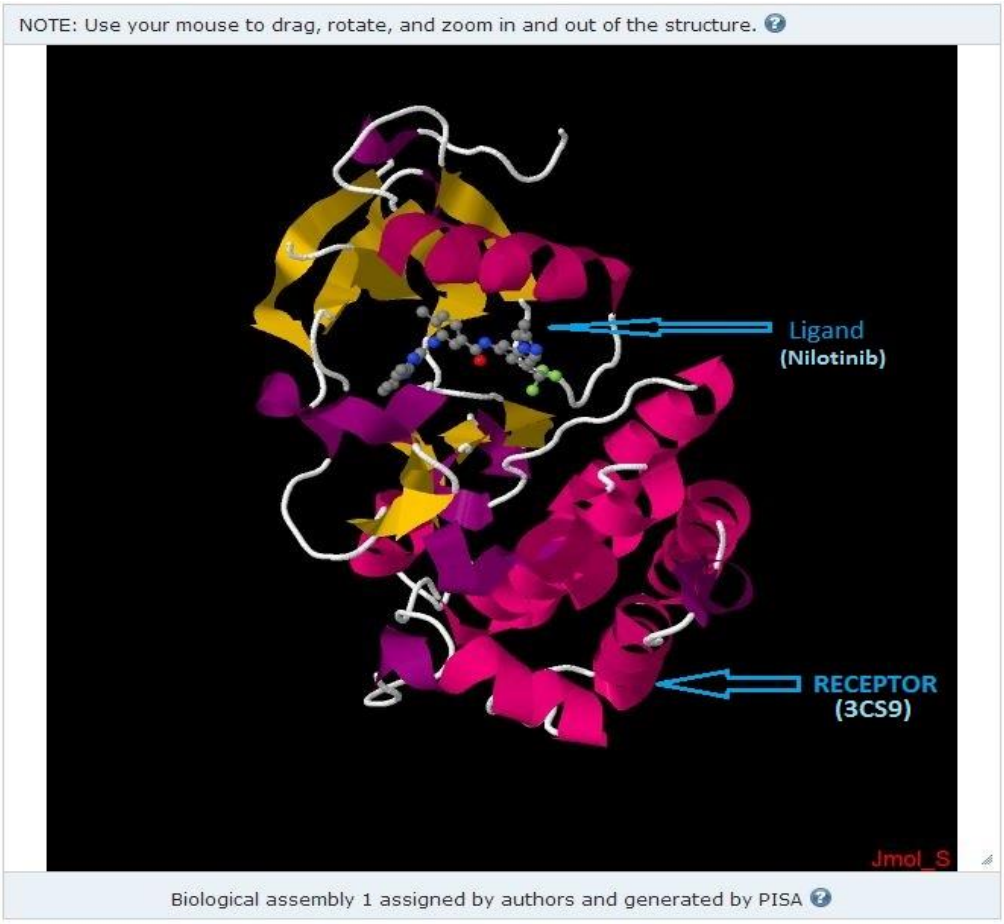


Figure 6. 3D view of 3CS9 (Human ABL kinase in complex with nilotinib) in Jmol_S.

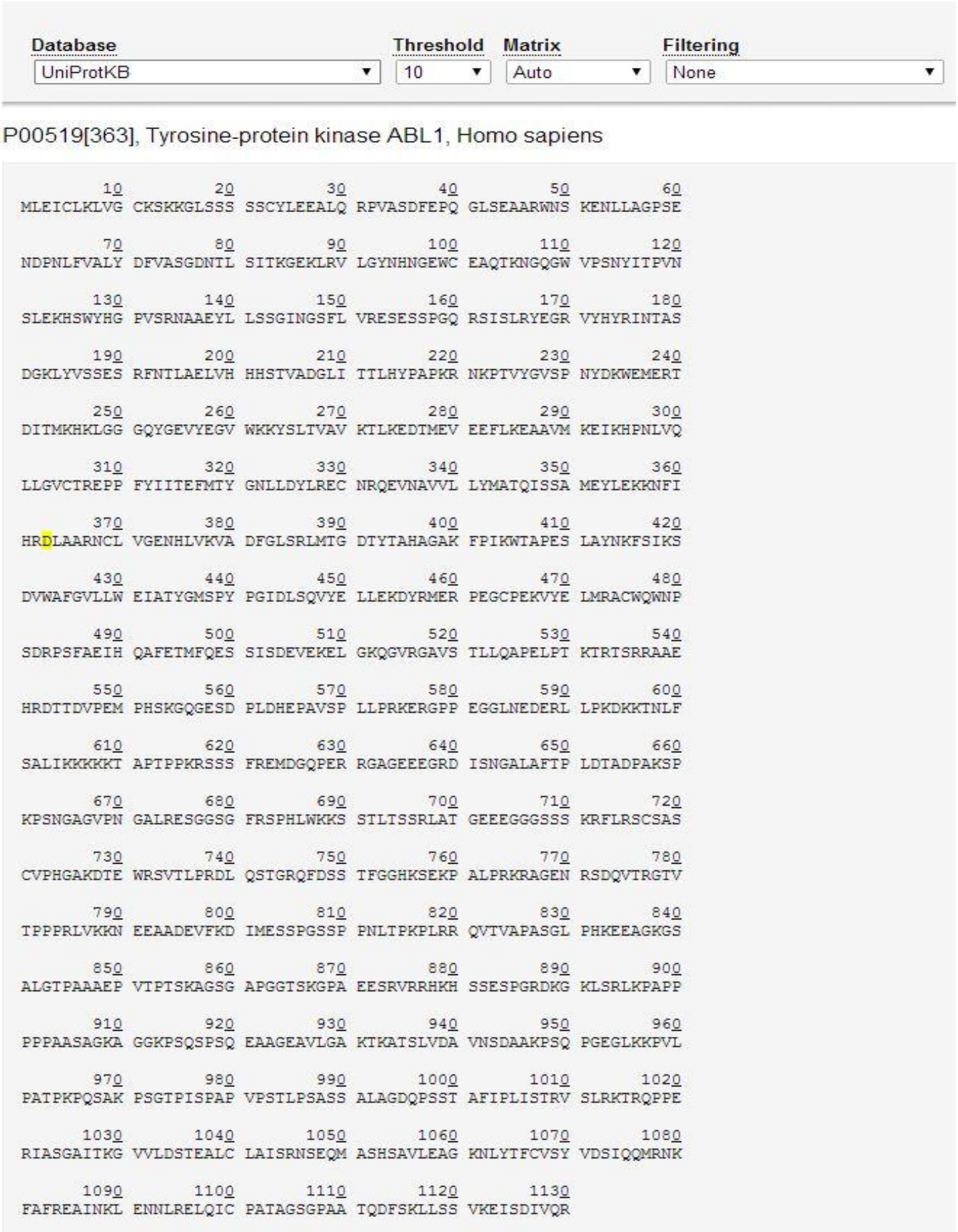


Figure 7. Snapshot of amino acid sequences of 3CS9 in UniProt.

4.3 RETRIEVING LIGANDS FROM PUBCHEM AND CONVERSION TO PDB FORMAT BY

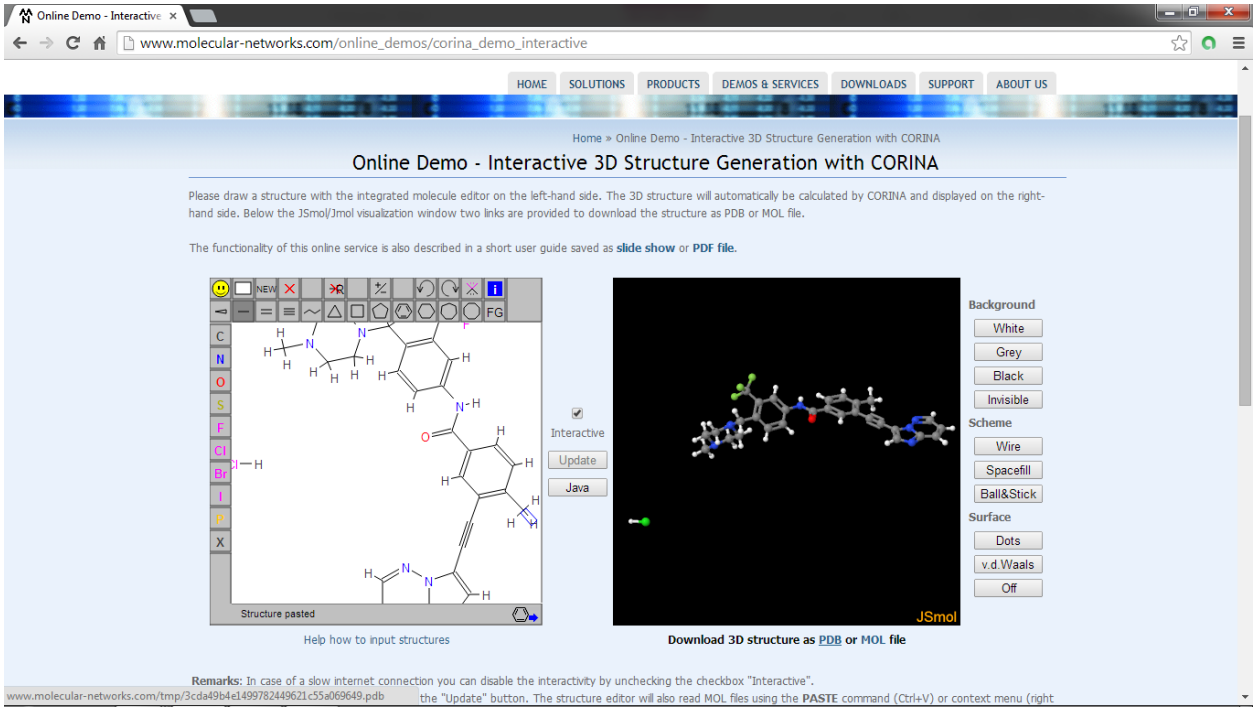


Figure 8. Snapshot of Corina Online 3D Generation webpage.

4.3.1 CORINA

If the inhibitor is in 2d format, then convert it to 3d in corina by the following steps [23]:

- First open the corina online converter webpage by entering "http://www.molecular-networks.com/online_demos/corina_demo_interactive" in the address bar.
- Then enter the ligand in the .sdf or .mol format by right clicking on the left hand side corina window.
- After submitting the ligand, corresponding 3d molecule will be generated on the right hand side of window.
- We can download the 3d generated molecule in either .pdb or .mol format.

4.3.2 OPEN BABEL

- Open pubchem by entering pubchem.ncbi.nlm.nih.gov in the address bar of the search engine.
- In search type the name of inhibitor & enter.
- All the available ligands will appear, if something is missing search for the same in chemspider.
- Save the 3D .sdf format of the ligands from pubchem and chemspider.

- Give the above .sdf format in open babel software as input by browsing the file from computer and convert it to the .pdb format by selecting the output format.
- Now the pdb format ligand is in 3D structure that will be used in following steps.

4.4 ENERGY MINIMIZATION OF LIGANDS

Before binding energy is being calculated, structural inconsistencies are first corrected, by adding hydrogen atoms and associated atoms with parameters of force field. Energy minimization is used to compute the equilibrium configuration of ligand. It makes the ligand flexible so that ligand can properly orient itself on the receptor during docking. Energy minimization was done by PRODRG, an online server following the given steps-

4.4.1 PRODRG SERVER

- Open Prodrg beta server.
- Paste the pdb format of ligand in the space provided & run Prodrg.
- Save the energy minimized pdb file (all hydrogens) generated by the server for future use.

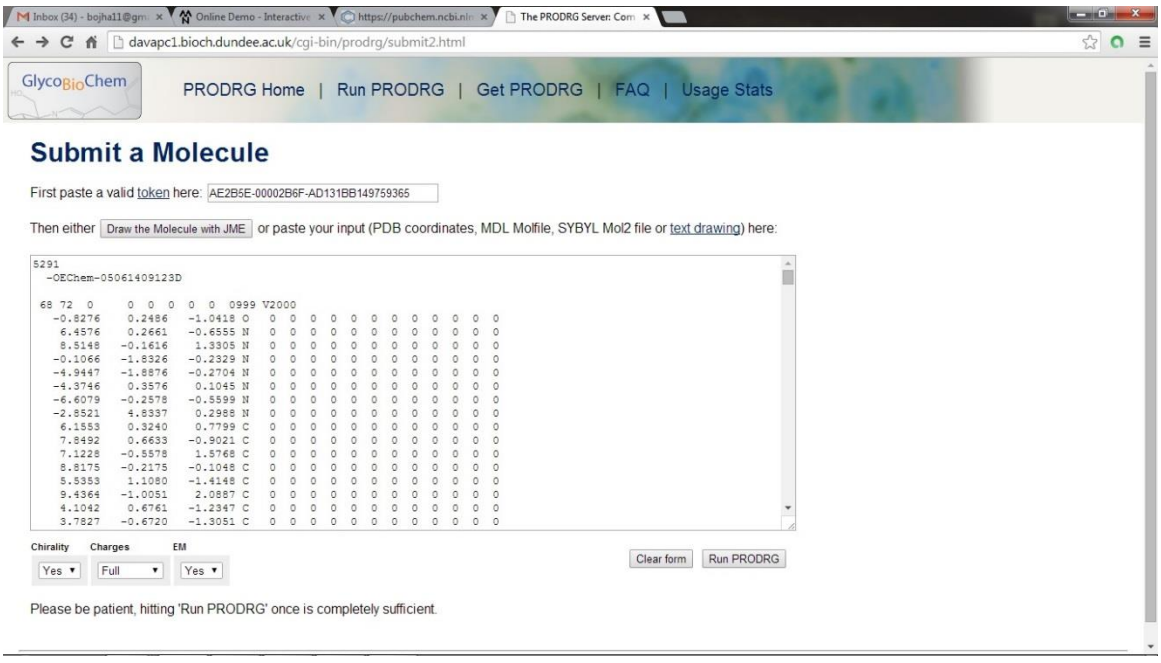


Figure 9. Snapshot of PRODRG server (online minimization page).

4.4.2 ARGUS LAB SOFTWARE

- Open the Argus lab software by double clicking on it.
- Open the ligand from file ⟹ open, then select the ligand.pdb molecule from the computer.

- From Calculation ⟹ Optimize Geometry, then a new window will pop up.
- Select UFF in Molecular Modelling (MM) of Hamiltonian window and Steepest Descent in Line Search of Geometry Search window.
- Then set Maximum Steps Taken as 1 lakh and click ok.
- After sometime the result should appear as "Geometry Optimization Converged".
- Then save the molecule as pdb.

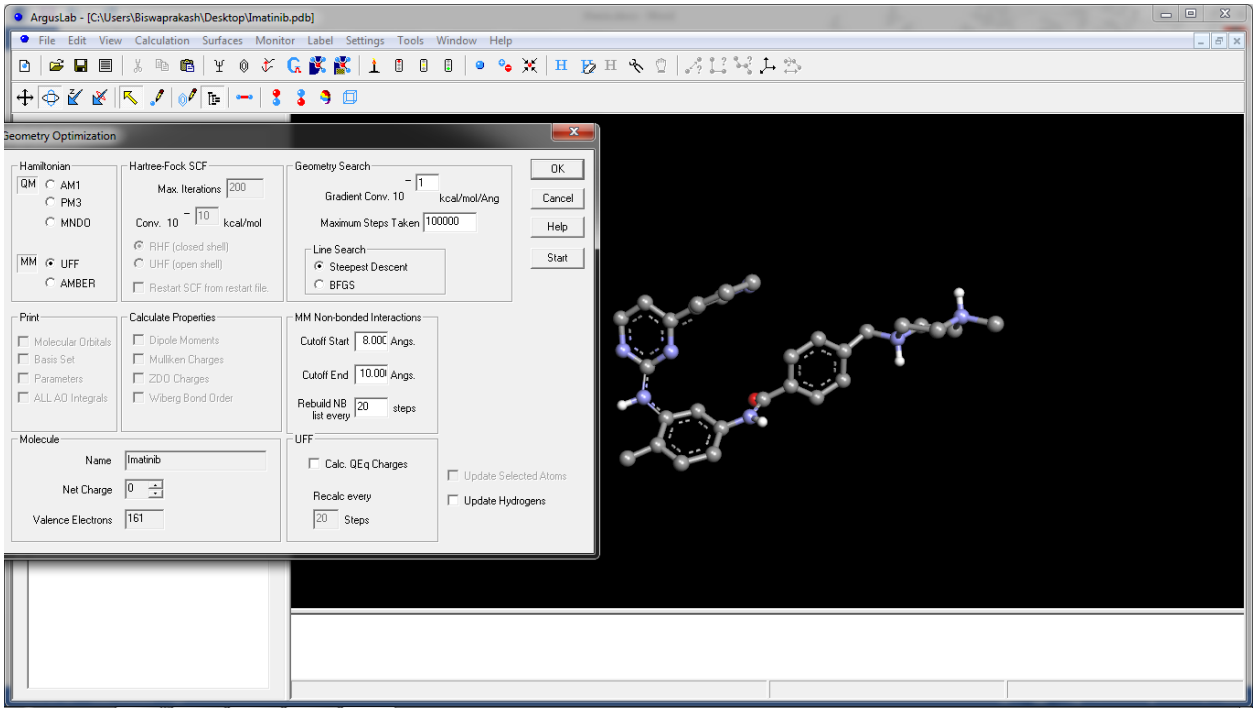


Figure 10. Snapshot of Energy minimization in Argus Lab software.

4.5 ENERGY MINIMIZATION OF RECEPTOR

The receptors obtained from the Protein Data Bank are generally complex files i.e., NMR structures of already docked receptor with some existing inhibitors. For using the same receptor for our docking studies, the receptor should be cleaned by eliminating the existing ligand and water molecules. The reactive groups created by removing this ligand should be substituted with hydrogens. For the cleaning of receptor Argus lab software has been used and for removal of the ligands CHIMERA has been used. The resultant PDB structure can be saved. The following steps are followed-

4.5.1 ARGUSLAB SOFTWARE

- Open the ArgusLab software by double clicking on it.
- Open the receptor file from File ⟹ Open, select the molecule by browsing the computer.
- View the molecule hierarchy wise then delete the water and miscellaneous molecules from residues.
- Then save the molecule as pdb.

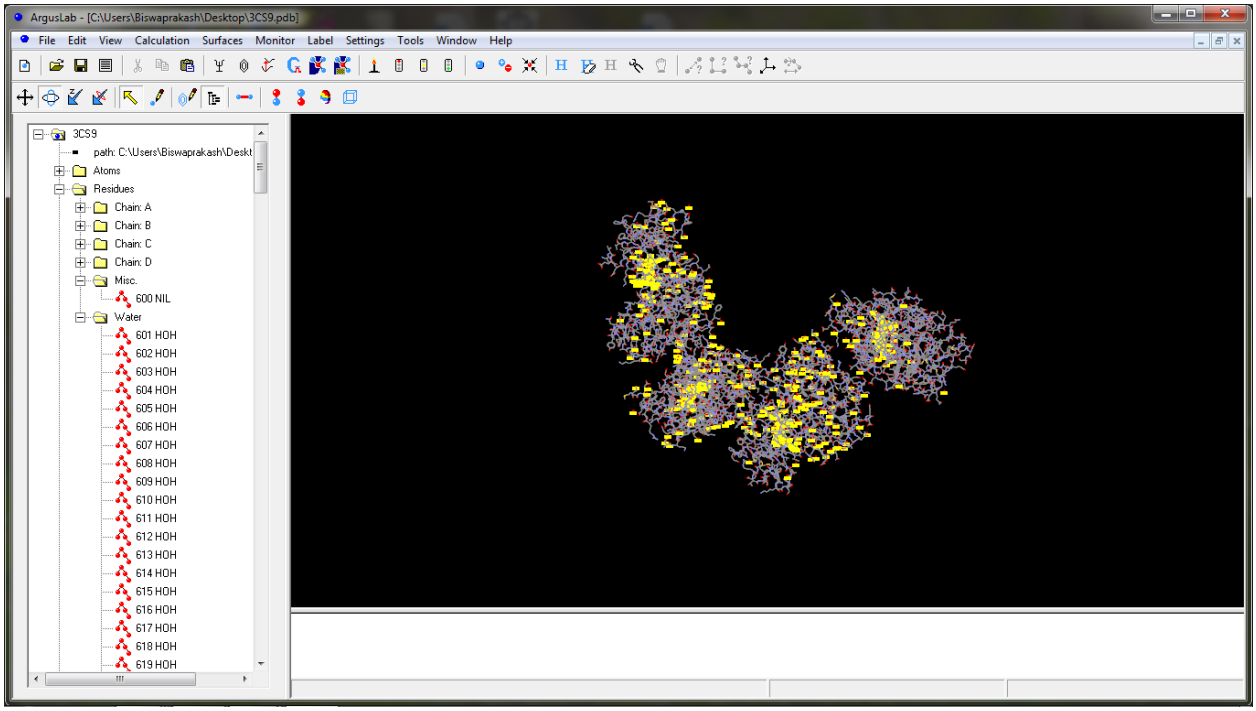


Figure 11. Snapshot of Receptor Cleaning in Argus Lab software.

4.5.2 CHIMERA

- Double click chimera open receptor file.
- Select ⟹ Structure ⟹ ligand.
- Delete the selected ligand from Action ⟹ Delete.
- Save the resultant file in pdb format and use for docking.

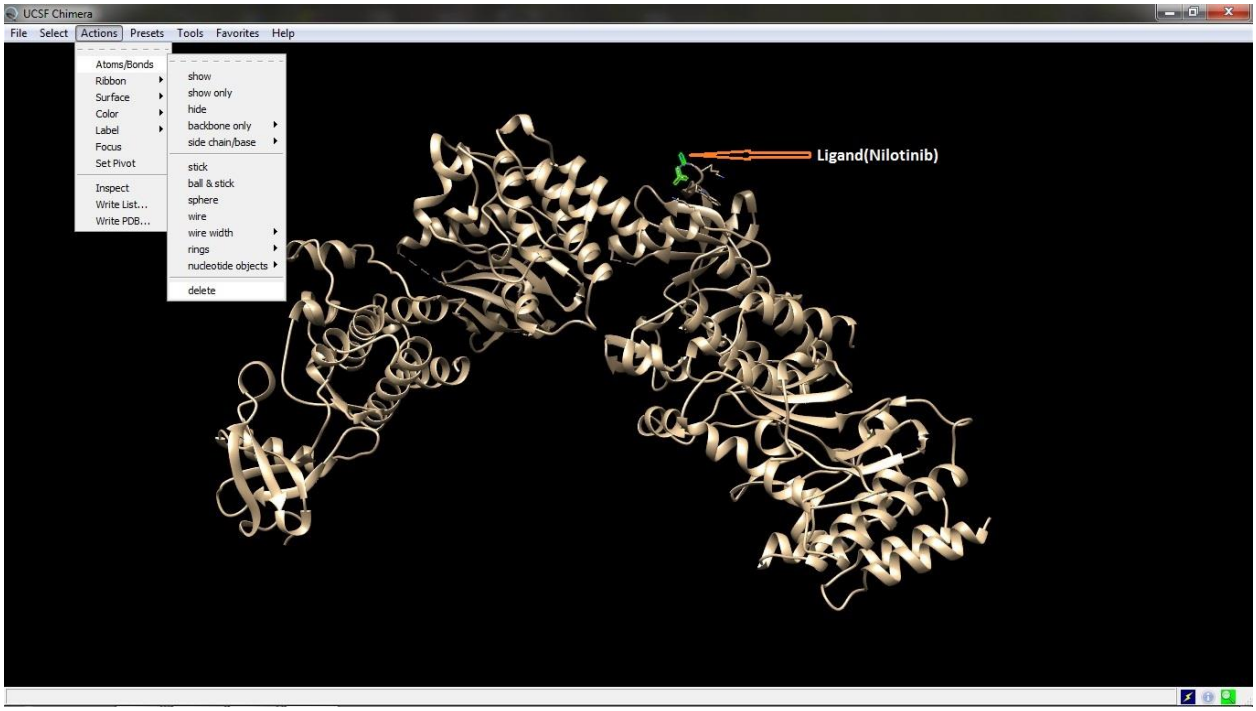


Figure 12. Snapshot of CHIMERA (Ligand Removal).

4.6 DOCKING BY AUTODOCK VINA 1.1.2

Docking is a method which predicts the binding affinity of a small molecule (ligand) with a larger molecule (receptor) when bound to each other to form a stable complex. Each docking program utilizes a unique scoring function to rapidly approximate properties such as receptor ligand binding. Autodock is a docking tool based on a linear regression analysis of free energy scoring function of how small a molecule is and the AMBER force field that occur between a ligand and protein target. It has an enhanced version i.e. Autodock Vina with an improved local search routine allowing the usage of multi-CPU or multicore computer setups. Autodock Vina is a modeling software for molecules and their structural simulation which is effective for docking. It is one of the most cited open-source program for doing molecular docking in the research community. Auto docking is a research centers for FightAIDS@Home project which is operated by World Community Grid [22].

The introduction of Autodock vina is due to the following features:

- It improves the average accuracy of binding predictions of molecules as compared to Autodock 4.
- In orders of magnitude, it is faster than AutoDock 4.

- Capable in reducing operation time, when operated in multiple CPUs or CPU cores on system.

After preparing the protein and ligand files through chimera and Argus lab software, all files must be kept in a single folder. Further modification to the protein and ligand, something like fixing the torsion residues etc. are made and the files are saved in PDBQT format. The conf.txt was prepared by putting the required grid size and coordinates of the active site. After docking completes, the results are saved in a file named log.txt in the folder.

4.6.1 PREPARATION OF PDBQT FILES

AutoDock 4 uses PBDQT file format for docking purpose. PDBQT files can be generated and viewed using MGL Tools. It acts as the input and output files as lock and key. The files are prepared for both ligand and receptor. Here the PDBQT files are generated in Autodock 4.

Receptor:

- Open the autodock 4 software by double clicking on it.
- From file ⟹ Read molecule ⟹ then choose the receptor molecule after cleaning from the computer.
- From Edit ⟹ Atoms ⟹ Assign AD4 type
- Again from Edit⟹ Charges⟹ Add Kollman Charges and repeat the step for Compute Gasteiger.
- Edit ⟹ Hydrogens ⟹ Add ⟹A new window will pop up and set the parameters as the default, then press Ok.
- From Grid ⟹ Macromolecule⟹ Choose, then a new window will appear, select the receptor molecule and press ok.
- After pressing ok, save the molecule as receptor.pdbqt appeared on the new window.

Ligand:

- Open the autodock 4 software by double clicking on it.
- From file ⟹ Read molecule ⟹ then choose the ligand molecule after energy minimization from the computer.
- From Ligand ⟹ Input ⟹ Choose, then a new window will appear, select the ligand molecule and press ok.

- From Ligand ⟹ Torsion Free ⟹ select Choose Root and repeat the same step for Detect Root.
- From Ligand ⟹ Output ⟹ Save as Pdbqt.

After preparation of the pdbqt files, the files are kept in a single folder in which other pdb files are kept and the docking procedure starts.

Docking Command:

For docking five files (i.e conf.txt, ligand.pdbqt, ligand.pdb, receptor.pdbqt and receptor.pdb) are required in a single folder and the steps are given below:

- Start the computer ⟹ click start menu ⟹ type cmd in the search menu in windows 7.
- Asses the folder in which the files are kept by giving the required command (cd c:\vina).
- Open the folder of the installed autodcok vina from program files(x86) from the computer.
- Then drag the vina.exe file to the command prompt menu from the vina folder of The Scripps Research Institute folder.
- Give the command as " __config conf.txt __log log.txt" to run the operation.

The steps followed for docking are mentioned below-

- Get the receptor (3CS9) coordinates (i.e. 22.328, 47.929, and 105.134) from the PDB by opening the pdb file in word and noting down the coordinates in the position of 363(Active site in uniprot id-P00519).
- Clean the receptor and remove the ligand.
- Add hydrogen atoms or residual atoms and minimize the receptor.
- Get the minimized ligand from PRODRG server or Argus lab software.
- Prepare the docking suitable files for LOCK and KEY (pdbqt files).
- Prepare the needing files for docking (conf.txt).
- Run the docking.
- Get the docking results from the file log.txt.

Conf.txt generation:

The contents of a sample conf.txt file are:

receptor = 3cs9.pdbqt

ligand = ligand.pdbqt

center_x = 22.328

center_y = 47.929

center_z = 105.134

size_x = 126

size_y = 126

size_z = 126

out = all.pdbqt

The grid center coordinates are (22.328, 47.929, 105.134).

4.7 ANALYZING DOCKING RESULTS

Primarily the results of AutoDock can be analyzed based on the best and lowest binding energies stored as a table in conf.txt file (Docking Log file). This file can be opened using text editor and can be investigated for the binding energy values. The interactions of the ligands with different amino acid residues of the receptor can found in detail by analyzing the complex.pdb using ligplot. Pymol is used for the preparation of the complex.pdb file but later interaction can be visualized by pymol in ligplot.

4.7.1 PYMOL

- Convert the all.pdbqt output file from vina folder to .pdb file format and save it.
- Open the all.pdb file from the folder in pymol viewer by browsing from the computer.
- Again open the receptor (3cs9.pdb) file in pymol from the vina folder.
- Now we can see the 9 posed positions of the receptor and the complex structure.
- Save both file as .pdb by going to file and clicking on save molecule.

4.7.2 LIGPLOT

- Lunch ligplot software.
- Open complex file which is saved by pymol viewer.
- Go to file and browse the file and click ok to view the interaction.
- Then a new window will ask the permission of running the ligplot for operation of visualization.

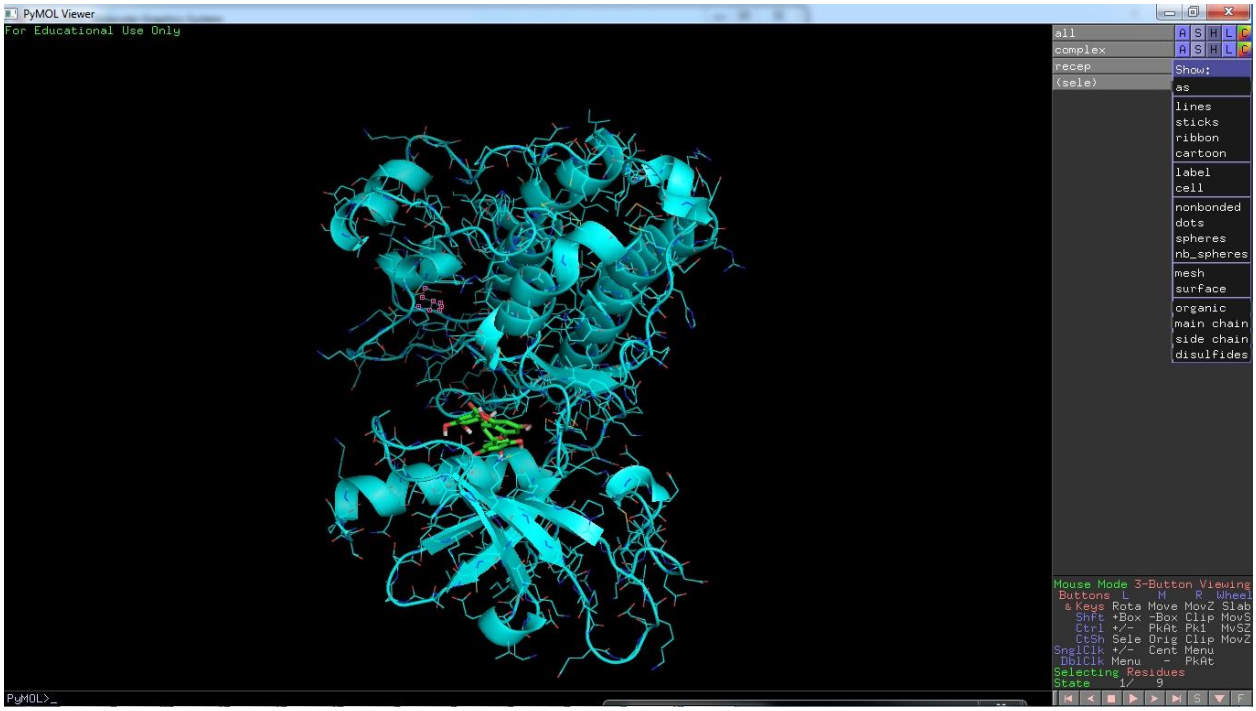


Figure 13. Posed position of the complex structure with receptor.

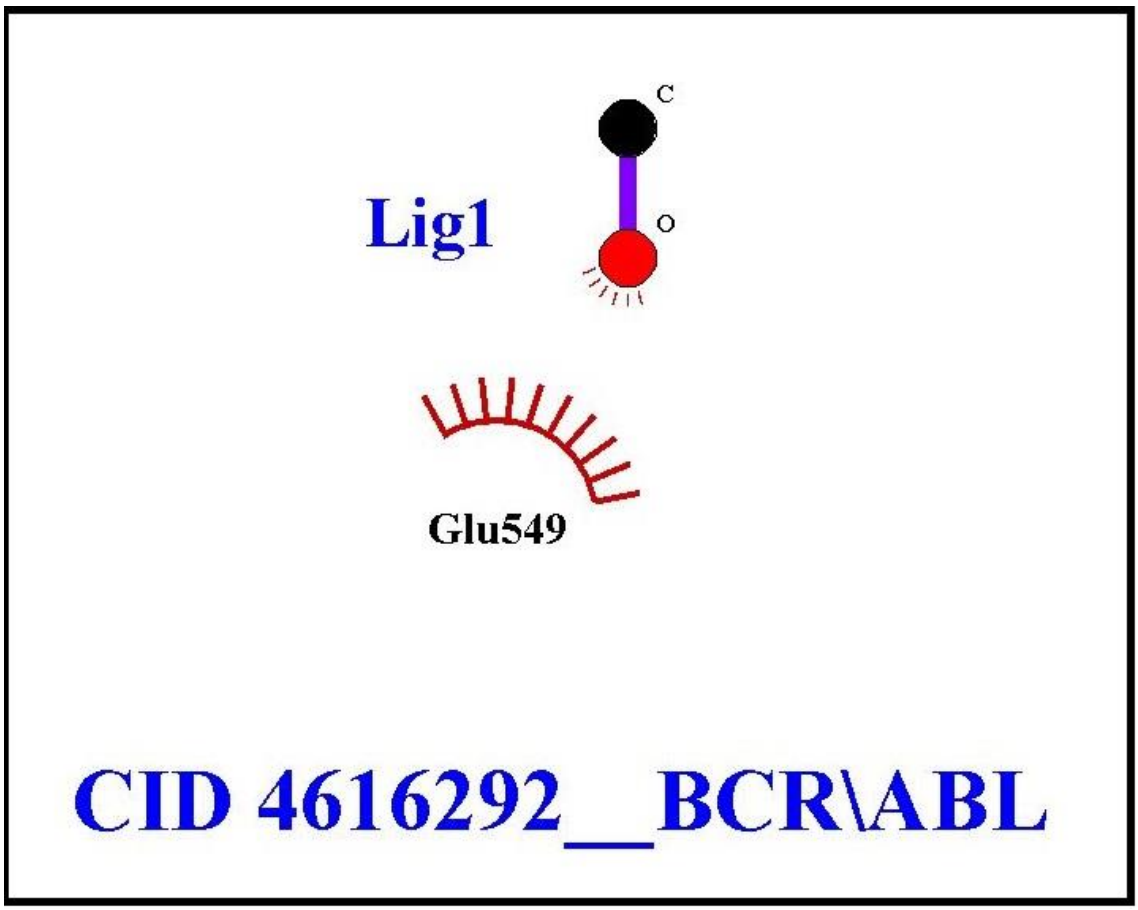


Figure 14. Atomic interactions of BCR ABL with CID 4616292.

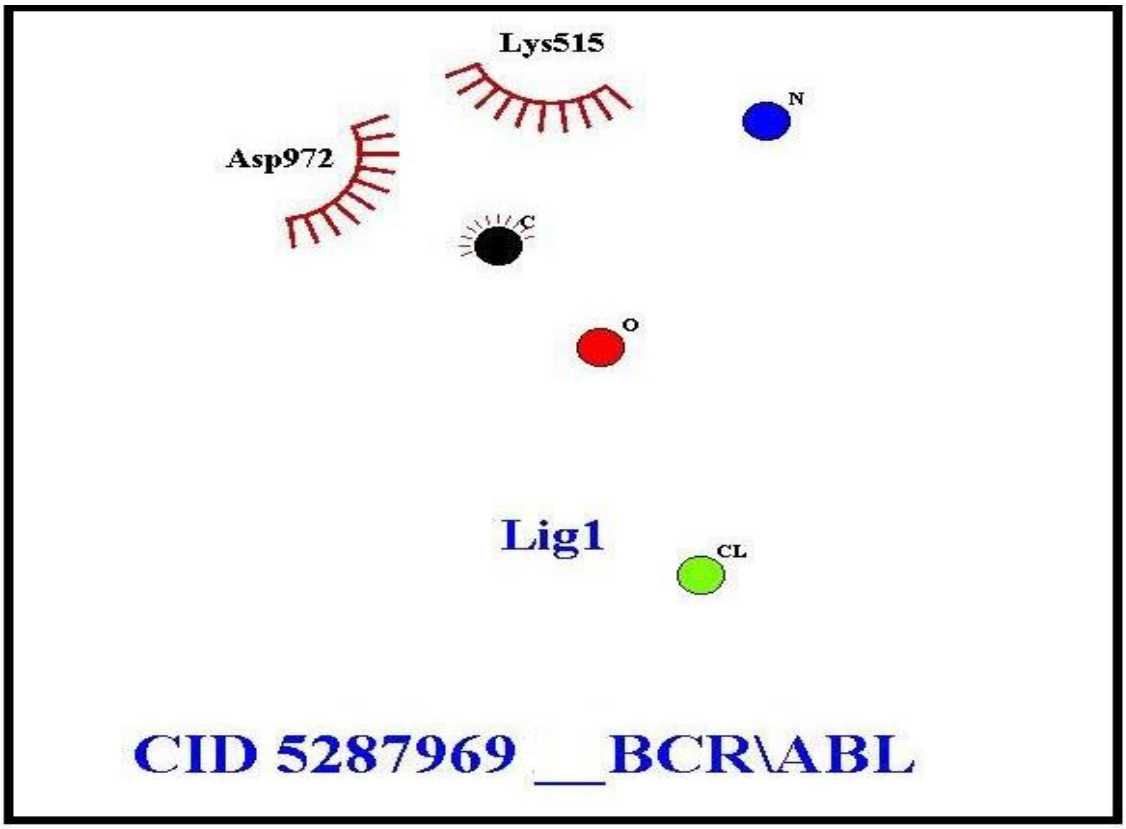


Figure 15. Atomic interactions of BCR ABL with CID 5287969.

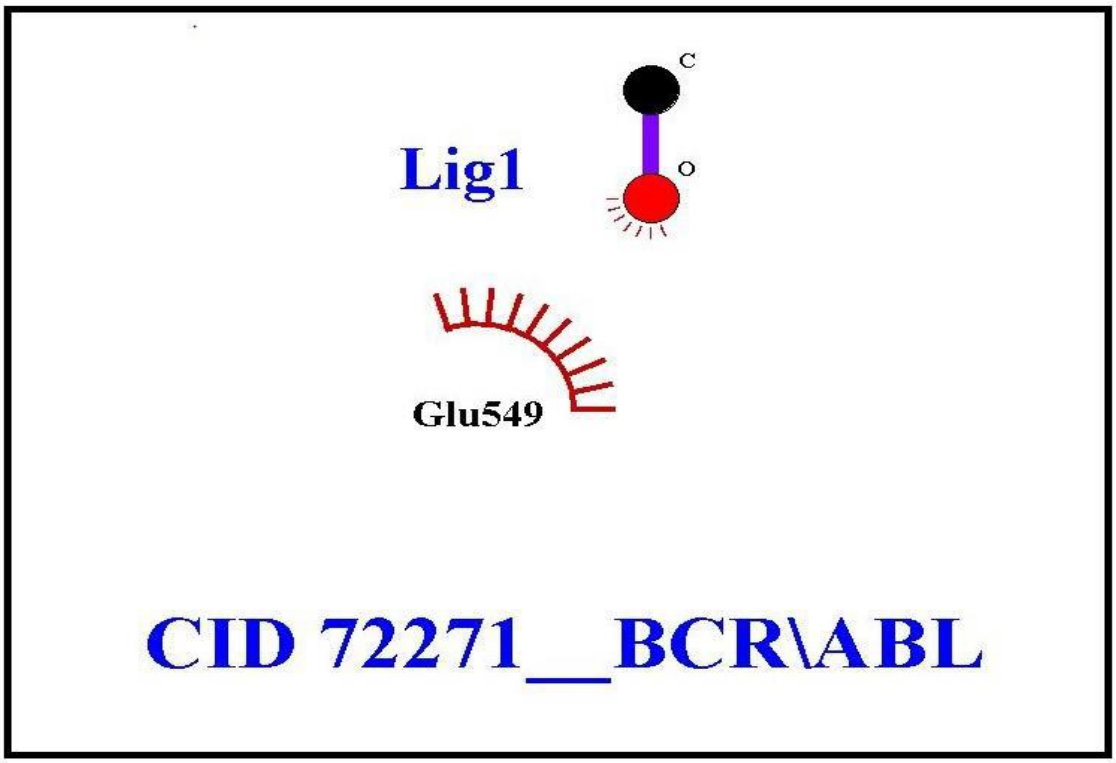


Figure 16. Atomic interactions of BCR ABL with CID 72271.

4.8 ADMET STUDIES

The success of a drug depend on its ADME (Absorption, Distribution, Metabolism and Elimination) characters in the body. The early prediction of ADME toxicity for drug likeness of a new ligand helps in reducing the probability of its failure at the drug development stage. Thus, *In silico* analysis like ADME prediction, Molecular descriptors calculation, Drug likeness prediction, Drug toxicity prediction can be done by the PreADMET tool. The steps involved in ADMET studies are as follows-

- Open FAF-Drugs homepage by typing: http://bioserv.rpbs.univ-paris-diderot.fr/FAF-Drugs/
- Then click on run FAF-Drugs option below.
- A new window will pop up. Then submit the .sdf file of the ligands for analysis by clicking upload menu in the new window.
- XLOGP3 was set as logP computation program.
- Rests of the filtering options were kept as default.
- Then click on the run button to start the processing.
- After the processing the overview page will open. Click on full screen to get the result of the drug.
- Click on ligand id, then tabular result will open, there we can analyze the Drug likeness, ADME and Toxicity of the ligand from the different images.

Pharmacokinetic properties of the protein kinase inhibitors were predicted by using FAF-Drugs2 server. ADME-Tox prediction helps in differentiating drug non like and drug like properties of docked molecules and predicts high probability of drugs failure or success rate due to its toxicity. This early *in silico* prediction helps in early preclinical assessment and thereby avoiding costly late stage preclinical and clinical failures [21].

ADMET Profiling can be categorized into ways;

1. **Oral Bioavailability:** Lipinski RO5, Veber Rule, Egan Rule, Bayer Oral Physchem score
2. **Drug Safety Profiling:** GSK 4/400 Rule, Pfizer 3/75 Rule, Phospholipidosis, Lilly MedChem Rules

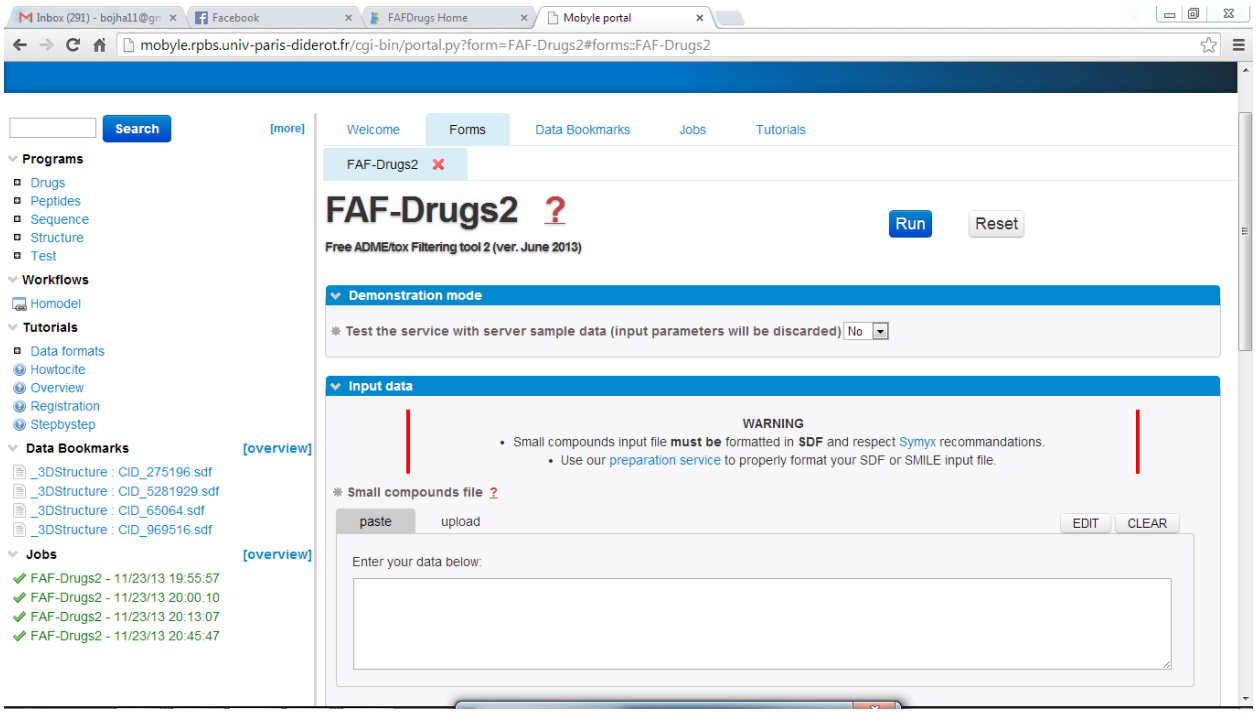


Figure 17. FAF-Drug server home page.

	Rule of 3	Rule of 5	*Drug-Like Soft* [1]	*Lead-Like Soft* [2]	R.E.O.S	ZINC	CNS	Respiratory
MW	≤ 300	*(≤ 500)*	100 - 600	150 - 400	200 - 500	60 - 600	135 - 582	240 - 520
logP	-3 to 3	*(≤ 5)*	-3 to 6	-3 to 4	-5 to 5	-4 to 6	-0.2 to 6.1	-2 to 4.7
HBA	≤ 3	*(≤ 10)*	≤ 12	≤ 7	≤ 10	≤ 11	≤ 5	-
HBD	≤ 3	*(≤ 5)*	≤ 5	≤ 4	≤ 5	≤ 6	≤ 3	-
HBonds	-	-	-	-	-	-	-	6 - 12
tPSA	≤ 60	-	≤ 180	≤ 160	≤ 150	≤ 150	3 - 118	51 - 135
Rotatable Bonds	≤ 3	-	≤ 11	≤ 9	≤ 8	≤ 12	-	3 - 8
Rigid Bonds	-	-	≤ 30	≤ 30		≤ 50		
Rings	-	-	≤ 6	≤ 4	-	≤ 7	-	1 - 5
MaxSizeSystemRing	-	-	≤ 18	≤ 18	-	≤ 12	-	-
Carbons	-	-	3 - 35	3 - 35	-	≥ 3	-	-
HeteroAtoms	-	-	1 - 15	1 - 15	-	≥ 0	-	-
H/C Ratio	-	-	0.1 to 1.1	0.1 to 1.1		≤ 2.0	-	-
Charges	-	-	≤ 3	≤ 3	-	≤ 3	-	-
TotalCharge	-	-	-2 to 2	-2 to 2	-2 to 2	-2 to 2	-	-
RO5 Violations	-	2	-	-	-	-	-	-
StereoCenters	-	-	-	≤ 2	-	-	-	-
References	35	13	13,19,24,30,49	30,32,47,48	34	24	36	25

Figure 18. Snapshot of physico-chemical property filters used in FAFDrugs2 server.

CHAPTER 5: RESULT AND DISCUSSION

5.1 RESULTS OF DOCKED INHIBITORS

Drug bank (Pubchem) is the source of newly synthesized molecules with unknown activity. We have downloaded a total 53 protein kinase inhibitors, structurally similar to Imatinib from drug bank for docking with BCR-ABL receptor. The Ligands and the receptors were retrieved and minimized as a preparatory step for Docking. Molecular Docking of ligand inhibitors are performed using Autodock vina. The BCR-ABL receptor is docked with the imatinib analogues which gave binding energy in the range of -3 to -9.1 Kcal/mol. The docking results are compared with a control drug Imatinib of binding energy of -8.2 Kcal/mol. Then the ligand are screened on the basis of their docked binding energies which is given in the Table. 4.
The results of binding affinity of 53 ligands with the receptor are tabulated below-

Table 4. Docking results of 53 Drug bank molecules with BCR ABL.

SL NO	LIGAND ID	BINDING ENERGY(-Ve) (Kcal/Mol)
1	CID 71300928	6.2
2	CID 56603728	7.2
3	CID 56671814	7.7
4	CID 51340302	7.4
5	CID 46908927	7.9
6	CID 22386467	8.3
7	CID 16760499	6.8
8	CID 16219471	6.9
9	CID 11957580	6.5
10	CID 11957465	6.0
11	CID 11679357	5.6

12	CID 11626560	7.5
13	CID 11557040	1.6
14	CID 9941095	6.4
15	CID 9910986	7.5
16	CID 9886292	7.8
17	CID 6858240	6.0
18	CID 5702541	7.0
19	CID 5702541	6.7
20	CID 5329098	8.0
21	CID 5312122	7.6
22	CID 5287969	9.1
23	CID 5280961	6.4
24	CID 5235506	7.8
25	CID 4616292	9.1
26	CID 3078519	7.4
27	CID 3062316	6.9
28	CID 3035414	5.3
29	CID 449241	6.8
30	CID 406563	6.9
31	CID 312145	7.2
32	CID 216239	8.6
33	CID 208909	1.9
34	CID 208908	7.8
35	CID 176871	7.0
36	CID 176870	6.5

37	CID 163751	6.6
38	CID 160355	7.2
39	CID 151194	7.3
40	CID 151193	4.0
41	CID 123631	8.2
42	CID 123596	3.3
43	CID 108152	7.4
44	CID 72271	9.1
45	CID 5291	8.2
46	CID 3843	8.4
47	CID 3547	7.2
48	CID 3546	6.5
49	CID 3544	5.9
50	CID 3499	8.6
51	CID 3220	7.4
52	CID 3134	4.8
53	CID 1352	7.0

Table 5. Ligand Screened for further ADMET studies.

SL NO	PUBCHEM ID	LIGAND NAME	Binding Energy(-Ve) (Kcal/Mol)
1	CID 72271	7-hydroxystaurosporine(UCN-01)	9.1
2	CID 5287969	Alvocidib	9.1
3	CID 4616292	7-hydroxystaurosporine(UCN-02)	9.1
4	CID 216239	Sorafenib	8.6
5	CID 3499	Go 6983	8.6
6	CID 3843	KT 5823	8.4
7	CID 22386467	Vatalanib	8.3
8	CID 123631	Gefitinib	8.2
9	CID 5291	Imatinib(Control drug)	8.2

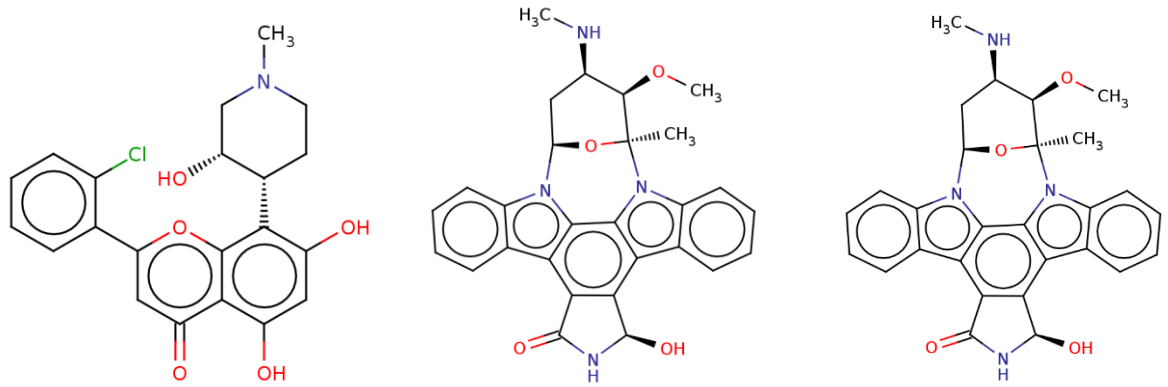
5.2 PreADMET RESULTS

Based on the binding energy of inhibitors with BCR ABL and structure of the compound, 9 ligands are selected for ADMET studies to know their Molecular descriptions, Drug Likeness, ADME data, Toxicity. Out of 9 inhibitors only 3 qualified as drug. But CID 72271and CID 4316292 are two molecules of same molecular weight with different structure as these are imatinib related protein kinase inhibitors so we are taking CID 72271 in to consideration. The results and the properties of the different ligands are shown below –

Table 6. *In silico* ADMET screening results of protein kinase inhibitors.

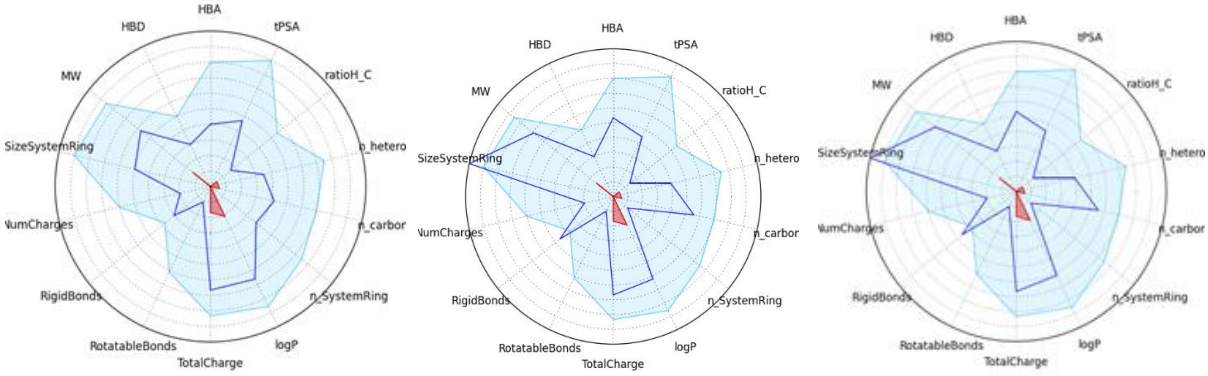
Ligand	LogP	LogSw	HBonds	Solubility (mg/l)	Oral Bioavailability (VEBER)	Oral Bioavailability (EGAN)	Phospholipidosis	Status
CID 72271	2.69	-4.63	8	4692.41	Good	Good	Noninducer	Accepted
CID 5287969	3.25	-4.49	6	4492.81	Good	Good	Noninducer	Accepted
CID 4616292	2.69	-4.63	8	4692.41	Good	Good	Noninducer	Accepted
CID 216239	4.07	-5.17	7	2648.90	Good	Good	Noninducer	Accepted
CID 3499	3.08	-4.29	7	6078.39	Good	Good	Noninducer	Accepted
CID 3843	3.50	-5.16	8	2850.94	Good	Good	Noninducer	Accepted
CID 22386467	4.48	-4.96	4	2442.80	Good	Good	Noninducer	Accepted
CID 123631	4.11	-4.89	7	3370.20	Good	Good	Noninducer	Accepted
CID 5291 (Control)	3.52	-4.92	8	3613.29	Good	Good	Noninducer	Accepted

Diferent properties of the compound are shown in tabular manner below:



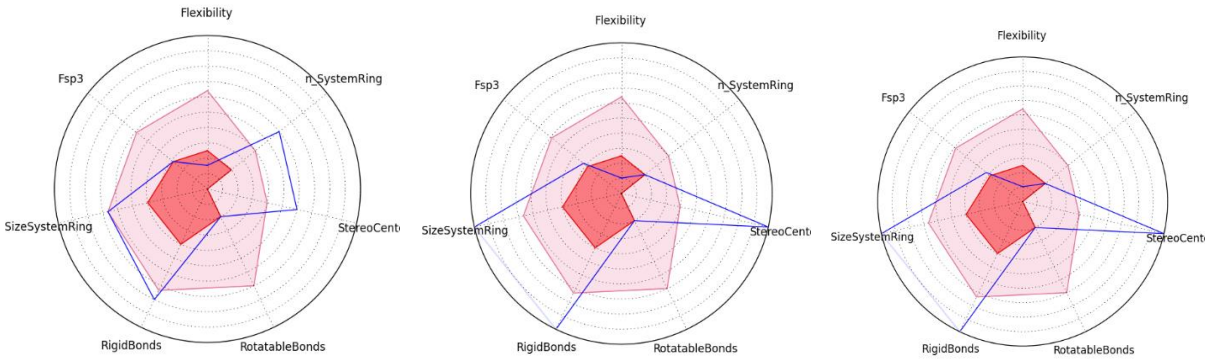
(a) (b) (c)

Figure 19. 2D Structure of (a) CID 5287969 (b) CID 4616292 (c) CID 72271



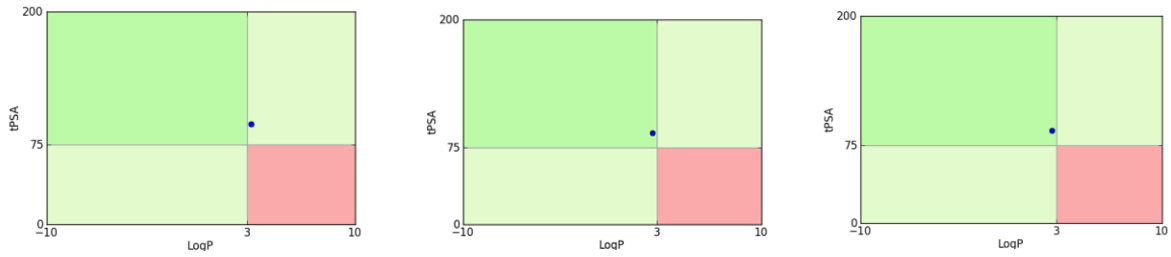
(a) (b) (c)

Figure 20. PhysChem Filter Positioning of (a) CID 5287969 (b) CID 4616292 (c) CID 72271



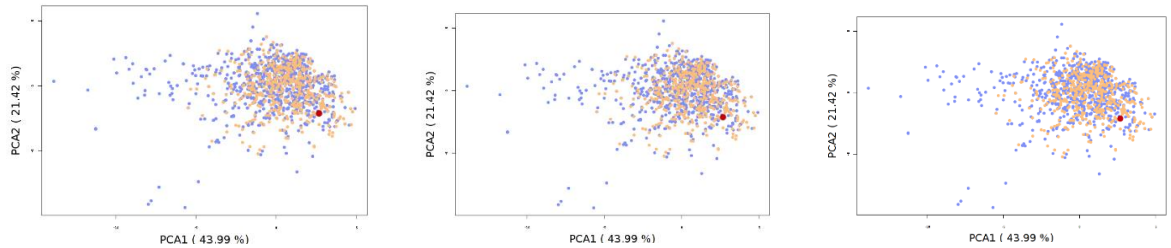
(a) (b) (c)

Figure 21. Compound Complexicity of (a) CID 5287969 (b) CID 4616292 (c) CID 72271



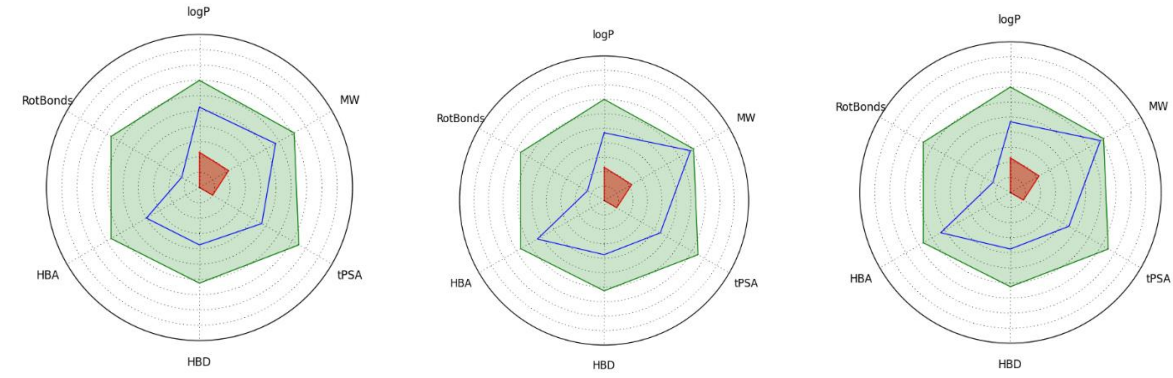
(a) (b) (c)

Figure 22. Pfizer 3/75 Rule Positioning of (a) CID 5287969 (b) CID 4616292 (c) CID 72271



(a) (b) (c)

Figure 23. Oral Property Space of (a) CID 4616292 (b) CID 72271 (c) CID 5287969



(a) (b) (c)

Figure 24. Oral Absorption Estimation of (a) CID 5287969 (b) CID 4616292 (c) CID 72271

CHAPTER 6: CONCLUSION

By analyzing the Molecular Docking results i.e, binding energies of all the inhibitory ligands, interactions of inhibitors with the receptor and the results from the ADMET studies, Alvocidib (CID 5287969) possesses promising inhibitory activity against BCR-ABL receptor. Hence, it can be concluded that Imatinib related protein kinase inhibitor Alvocidib can effectively inhibit the pathway initiated by BCR-ABL leading to CML. Again, Alvocidib can also prove to be a safe drug for oral medication. However, the results need to be validated by *in vitro* followed by clinical trials on CML patients.

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